TOXICOLOGIST'S REVIEW

PLA#: 97-0501
SPONSOR: CHIRON CORPORATION (b)(4)
PRODUCT: recombinant, human interleukin-2 (Proleukin®)
FORMULATION: lyophilized
RELATED DOCUMENTS: [redacted] PLA #88-0660
PROPOSED INDICATION: metastatic melanoma
ABBREVIATIONS: rhIL-2, recombinant, human interleukin-2, Proleukin®; HSA, human serum albumin; NOAEL, no observable adverse effect level; CTL, cytotoxic T lymphocyte(s); LD_{10}, dose at which lethality is observed in 10% of the animals; D5W, 5% dextrose solution in water; ANOVA, analysis of variance; AUC, area under the concentration vs. time curve

received 4/10/97; assigned 4/26/97; completed 1/7/98

ABSTRACT:

PROLEUKIN® (interleukin-2, rhIL-2) is a potent stimulator of T cell proliferation and activation of the immune response. In murine models of human metastatic melanoma, PROLEUKIN® is pharmacologically active at doses greater than or equal to 0.4 mg/kg/d, administered daily by i/v injection for 4 to 10 d. Dilution of PROLEUKIN® in vehicle containing 0.1% HSA had no significant effect on the anti-tumor activity of the agent. Dose- and schedule-related increases in mortality after PROLEUKIN® treatment were observed in mice administered 10 or 15 mg/kg/d for 7 to 14 d, which were further increased by the addition of HSA. Similar findings of increased toxicity and mortality were also observed in preclinical toxicity studies in rats treated with repeated doses of rhIL-2 treatment administered by i/v injection for 14 d, when HSA was present. Treatment-related toxicities included increases in total peripheral blood leukocyte, eosinophil and lymphocyte numbers, increases in total and indirect serum bilirubin and transaminase levels, and mortality. In outbred, Sprague-Dawley CD and inbred Fischer rats, no NOAEL could be determined. The lowest dose tested in CD rats was 0.5 mg/kg, and 2.0 mg/kg in the Fischer strain. Pharmacokinetic evaluation of PROLEUKIN® in these two strains of rats demonstrates that the presence of HSA in the diluent results in 23 to 35% increased in C_{max}, decreases in clearance and V_{dss}, and 40 to greater than 70% increases in total exposure, as defined by $AUC_{t0-\infty}$. Fifty to 200 percent greater values for $AUC_{t0-\infty}$ were achieved in Fischer rats as compared to CD rats when HSA was present, suggesting that the reasons for the increased mortalities observed in this strain using HSA in the diluent were related to the cumulative exposure to PROLEUKIN®. Direct comparison of a 5 min i/v infusion of 0.2 mg/kg PROLEUKIN® with the same dose delivered by i/v bolus injection demonstrated that the C_{max} and $AUC_{t0-\infty}$ values for IL-2 were increased approximately 3-fold in rats receiving the bolus injection as opposed to those animals treated by slow infusion. Taken together, these data demonstrate that the previous increases in mortality noted in the preclinical studies using PROLEUKIN® in rats and mice are related to the presence of HSA in the formulation, and the schedule of delivery of the biologic. These data support the proposed labeling for the use of PROLEUKIN® formulations diluted without HSA, and administered by slow i/v infusion over a 5 to 15 min time period.

INTRODUCTION:

Specific, anti-tumor immunity involves activation of multiple cell types in the immune system, the most efficient being cytolytic T lymphocytes. To induce specific, anti-tumor T lymphocyte-mediated immune response, recognition of not only the tumor antigen of interest, but also co-stimulatory interactions between specific ligands present on either the tumor cell or the antigen presenting cell, and the target T lymphocytes are required. This second, co-stimulatory signal may also be provided by soluble factors, such as cytokines or other peptide molecules which bind to specific, cell surface receptors and initiate various signal transduction pathways, resulting in augmentation of effector function.

Interleukin-2 is a T lymphocyte-derived cytokine which binds to specific receptors present on T cells and natural killer (NK) cells, and will activate them for tumor cytolysis, cytokine secretion, and other effector functions. Both $CD4^+$ and $CD8^+$ T lymphocytes express the receptor for rhIL-2, and develop increased cytolytic effector and cytokine synthetic function after exposure to the biologic. Preclinical studies in various murine tumor models have demonstrated that rhIL-2, when administered to tumor-bearing animals for periods of 10 to 14 days can result in regression of tumor burdens, long-term survival, and increased resistance to tumor rechallenge. Analysis of splenic lymphocytes obtained from these animals has shown that the anti-tumor effects are due at least in part to augmentation of cytolytic T cell function. This effect includes activation of both direct cytolysis of tumor cells by the CTL, as well an increased synthesis of other, T lymphocyte-derived cytokines, which may have further direct or indirect anti-tumor effects as well.

In the present submission, the sponsor is applying to expand the indication of the anti-tumor efficacy of PROLEUKIN® against metastatic melanoma. This tumor type responds poorly to conventional therapies, but in preclinical models has been demonstrated to respond favorably to repeated doses of PROLEUKIN®, resulting in increased long-term survival and immunity to tumor rechallenge. Data included in the clinical portion of the application have shown similar induction of specific, anti-tumor immunity in melanoma patients after treatment with repeated doses of PROLEUKIN®, with achievement of complete, durable remissions of tumor, lasting greater than 4 years in approximately 6% of the recipients.

Interleukin-2 is presently licensed for the treatment of metastatic renal cell carcinoma, at a dose of 600,000 IU/kg, administered by slow i/v infusion every 8 hours as tolerated, or to a maximum of 14 doses. The course is repeated after a nine day rest period for one complete cycle of treatment. In both melanoma and renal cell cancers, complete and durable responses were achieved with this dosing regimen.

PRECLINICAL TOXICOLOGY, PHARMACOLOGY AND BIODISTRIBUTION:

Comment: The preclinical data in the present submission include animal studies to address several of the phase 4 commitments from the original licensing application. There is no record of these data having previously been reviewed; therefore, the studies have been identified from the present submission and are reviewed below. Additionally, several study reports from preclinical safety and pharmacokinetic studies from the original application are also included in the submission, for informational and comparative purposes only. These data were reviewed at the time of the initial approval, and were not re-reviewed for the present application; a listing of the studies is provided following the reviews of the relevant studies.

Preclinical Pharmacology and Toxicology Study Summary:

1. Effects of human serum albumin on PROLEUKIN® IL-2 toxicity in BDF_1 mice in the day 3 artificial metastasis model. Study #B16-196 and #PH043093. BDF_1 mice, 10 ♀/group; vehicle controls, 10, 15 mg/kg/d x 10 PROLEUKIN® (lot #), +/- 0.1% HSA, non-GLP; Chiron Corporation, Emeryville, CA. **Volume 4, pp. 338-352.**

2. Comparison or the effects of HSA/PROLEUKIN®-IL2 in Fischer and CD rats: A dose-finding study. Study #PH72993. Specific pathogen-free CD rats (weight range 200-400 g, 8-10 weeks old), 3/sex/group; vehicle (0.1% HSA in D5W), 0.5, 1.0 mg/kg/d PROLEUKIN®, lot #LAP-2018 x 14 d, +/- 0.1% HSA, i/v; Fischer rats (weight range 150-200 g, 8-10 weeks old), 3/sex/group; vehicle, 2, 4, 8 mg/kg/d PROLEUKIN®, lot #LAP-2018 x 14 d, +/- 0.1% HSA, i/v; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 4, pp. 353-390.**

3. Effect of PROLEUKIN® IL-2 dosing duration on mortality in the day 3 B16 artificial metastasis model. Studies #B16-191 and B16-195. Final report dated 6/19/92; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 5, pp. 7-17.**

4. Effect of PROLEUKIN® dilution method on anti-tumor efficacy in the low dose day 1 B16 artificial metastasis model. Studies #B16-184, #B16-185, #B16-186, #B16-187, #B16-192, and #B16-193. Final report dated 6/22/92; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 5, pp. 18-56.**

Pharmacology and Toxicology Review:

Study #B16-196 and #PH043093. ***Effects of human serum albumin on PROLEUKIN® IL-2 toxicity in BDF_1 mice in the day 3 artificial metastasis model.***

The purpose of the present study was to compare the toxicities of PROLEUKIN® in tumor-bearing mice, when the biologic was formulated in either D5W or in vehicle containing 0.1% HSA. Hybrid, BDF_1 female mice were inoculated i/v with a sub-lethal dose of B16 melanoma tumor cells, and lung metastases were allowed to establish for 3 d. At that time, treatment with vehicle, 10, or 15 mg/kg PROLEUKIN® formulated with or without HSA was initiated. Because the doses of PROLEUKIN® used in the present study could not be further diluted from the reconstituted material without significant protein precipitation and loss of bioactive material, concentrated HSA (30 µl of a 25% sterile solution) was added to the syringes immediately prior to injection to obtain a final concentration of 2.5% HSA. Injections were administered daily by i/v bolus dosing, for 10 d. Animals were observed daily for signs of mortality, and the incidence compared between the different groups.

There were no deaths observed in mice treated with the excipient control, administered either with or without HSA. In the animals treated with 10 mg/kg PROLEUKIN®, mortality was noted in 1/10 mice treated with either of the PROLEUKIN® formulations. The number of deaths were increased at the higher dose in both groups as well; the incidence was 3/10 mice in the PROLEUKIN® alone group, and 5/10 animals receiving PROLEUKIN® administered with HSA. Although not statistically significant ($p = 0.93$, Kruskal-Wallace ANOVA), the addition of HSA did appear to be related to increased mortality at the higher dose group.

In conclusion, a dose-related increase in mortality was observed in B16 melanoma tumor-bearing mice after repeated i/v injection of PROLEUKIN® for 14 d. This effect was observed regardless of whether HSA was present in the formulation. Although not statistically significant, the addition of HSA did appear to be related to increased mortality at the 15 mg/kg/d dose group. This dose is approximately 15 times greater than the licensed human dose of 600,000 IU/kg PROLEUKIN® q 8 h.

Study #PH72993. ***Comparison or the effects of HSA/PROLEUKIN®-IL2 in Fischer and CD rats: A dose-finding study.***

A comparison of the toxicities and mortality related to treatment with PROLEUKIN® formulated with or without HSA was conducted in outbred Sprague-Dawley CD (CD) and inbred Fischer rats. Previous studies had demonstrated a difference in the susceptibility to the lethal effects of rhIL-2 between the two strains.

Age-matched, specific pathogen free CD and Fischer rats were used in the present study, and allowed to acclimate for 7 d prior to initiation of PROLEUKIN® treatment. PROLEUKIN® was reconstituted from the lyophilized, vialed material with 1 ml of WFI, then further diluted in D5W to reach the final concentrations needed for dosing. Because the doses of PROLEUKIN® used in the present study could not be further diluted from the reconstituted material without significant protein precipitation and loss of bioactive material, concentrated HSA was injected immediately prior to PROLEUKIN® through a three-way stopcock. Injections were administered daily by i/v bolus dosing, for 10 d. Animals were observed daily for signs of mortality, and the incidence compared between the different groups. Samples of peripheral blood from surviving animals in the control and high dose rhIL-2 groups only were analyzed at study termination for clinical pathology. No gross nor histologic evaluation of target tissues of rhIL-2 toxicity was performed.

Mortality data are demonstrated in the table below. Increases in deaths were observed in both strains of rats, which were related not only to the presence or absence of HSA, but to the dose of PROLEUKIN® administered as well. Fischer rats demonstrated higher incidences of mortality in both formulation groups than did the CD rats; however, the doses used in the Fischer strain were 4 to 16 times higher than those employed in the CD rats, and the results cannot be directly compared

TABLE I - Comparative Mortality in PROLEUKIN®-Treated CD and Fischer Rats

CD Rats			Fischer Rats		
Dose	**D5W only**	**D5W + 0.1% HSA**	**Dose**	**D5W only**	**D5W + 0.1% HSA**
0.5 mg/kg	0/6	2/7 (28.6%)	2.0 mg/kg	1/6 (16.7%)	5/6 (83.3 %)
1.0 mg/kg	0/6	2/6 (33.3 %)	4.0 mg/kg	3/6 (50%)	6/6 (100%)
			8.0 mg/kg	6/6 (100%)	6/6 (100%)

Comment: The sponsor states in the summary of this study report that Fischer rats were previously shown to be more "resistant" to the toxicities of rhIL-2, and therefore higher doses were used in the present study.

Comment: The incidence of mortality noted in the CD rats is similar to that previously reported in the original licensing application for PROLEUKIN® (PLA #88-0660, study #I01-91-007). In that study, 1/20 and 2/20 deaths were noted in CD rats treated with 0.5 and 1.0 mg/kg/d rhIL-2, respectively, formulated without HSA. The incidence of mortality was increased to 4/20 (25%) and 11/20 (55%) at these same doses in animals receiving the HSA in conjunction with PROLEUKIN®.

The majority of deaths occurred fairly late during the treatment with rhIL-2 in the CD rats (days 10, 12, 13, and 15). Similar times of death were noted for Fischer rats at the 2 mg/kg/d dose group with or without HSA; however, deaths occurred earlier in the two higher dose groups, and an apparent, although not statistically significant relationship between the early deaths and the presence of HSA was observed. At the highest dose group, in which no animals survived to study termination with either rhIL-2 formulation, deaths occurred at days 6, 8 (2 rats), 9 (2 rats), and 11 in the group treated with PROLEUKIN® in D5W alone, and at days 6, 7 (3 rats), 8, and 9 in animals treated concomitantly with rhIL-2 and HSA.

Significant differences in total leukocyte, lymphocyte, and platelet counts, red cell numbers, MCV and MCH were observed between the CD and Fischer rats strains prior to initiation of dosing with PROLEUKIN®. All of the Fischer rats treated with the 8 mg/kg/d dose of PROLEUKIN® died prior to scheduled sacrifice, so clinical pathology data are not available for this strain. In the surviving CD animals at study termination, serum BUN, globulin, glucose, and bicarbonate levels were significantly increased from baseline after treatment with either formulation of PROLEUKIN®. Two to three-fold increases in peripheral blood eosinophil counts were also noted in both of these dose groups. Additionally, significant ($p \leq 0.01$) increases in ALT and total and indirect bilirubin were noted in the CD rats treated with PROLEUKIN® and HSA. A 1685% increase in the mean value for total bilirubin as compared to baseline was noted in this group; mean total and indirect bilirubin values were also increased by approximately 3 to 5-fold in these animals as compared to rats treated with PROLEUKIN® diluted in D5W alone.

Taken together, these data demonstrate that the addition of HSA to the vehicle for delivering formulated PROLEUKIN® results in increased lethality in both Fischer and CD rat strains, and that this effect is also related to both the daily as well as the cumulative dose of rhIL-2 administered. No clinical pathology data for the lower dose groups were obtained, so it is not possible to define a true NOAEL for this study; however, the dose of PROLEUKIN® in CD rats formulated without HSA in which no mortalities occurred was 1.0 mg/kg/d, given by i/v injection for 14 d. Because mortalities were noted in all dose groups of Fischer rats and in both formulations, no NOAEL an be defined for this strain.

Studies #B16-191 and B16-195. ***Effect of PROLEUKIN® IL-2 dosing duration on mortality in the day 3 B16 artificial metastasis model.***

In the present study, the effects of duration of PROLEUKIN® treatment, when delivered without HSA were compared in B16 melanoma tumor-bearing mice. Syngeneic mice were inoculated by i/v injection with sub-lethal doses of B16-BL6 tumor cells on d 0, and lung metastases were allowed to establish for 3 d prior to initiation of PROLEUKIN® treatment. Groups of 10 mice each were treated daily by i/v injection with either 10 or 15 mg/kg/d for 10 or 14 days, or for 7 days with 15 mg/kg/d PROLEUKIN® diluted in D5W. Animals were observed daily for mortality, and the incidence of deaths in each treatment group noted is presented in the table below. No further evaluation of toxicity or gross or histopathology was conducted in this study.

TABLE II - Effect of Duration of PROLEUKIN® Treatment on Mortality in B6F1 Mice

	Dose of PROLEUKIN® Tested	
Duration of Treatment	**15 mg/kg/day**	**10 mg/kg/day**
7 days	- n.d.-	3/10
10 days	1/10	7/10
14 days	7/10	10/10

The incidence of deaths on study was increased in proportion to both the dose and the duration of PROLEUKIN® administration. At the 15 mg/kg/d dose, 3/10 mice died after 7 d of treatment, while treatment for 10 or 14 d resulted in 70 and 100% mortality incidences, respectively. By contrast, at the 10 mg/kg/d dose, only 1/10 animals died in the group treated with IL-2 for 10 d, and 7/10 animals died in the 14 d PROLEUKIN® treatment group.

These data further support the hypothesis that the toxicities of PROLEUKIN® are related to both the dose and schedule of administration, as has been previously described both in preclinical studies and in the clinical setting.

Comment: The sponsor states in the conclusion of the study report that this study was designed to define an optimal schedule for rhIL-2 administration in future animal experiments, and that the data support using a 10 d schedule of PROLEUKIN® as a means to increase the mortality to a level greater than or equal to an LD_{10}. Although preclinical doses for biologic agents, specifically cytokines are not usually defined by this means, this schedule does appear to be appropriate in terms of both defining expected toxicities as well as pharmacologic activity (please see study immediately below).

Studies #B16-184, #B16-185, #B16-186, #B16-187, #B16-192, and #B16-193. ***Effect of PROLEUKIN® dilution method on anti-tumor efficacy in the low dose day 1 B16 artificial metastasis model.***

The anti-tumor activity of rhIL-2 prepared and delivered with or without HSA was compared in B16 melanoma tumor-bearing mice to determine if the efficacy of PROLEUKIN® could potentially be altered by the excipients. Hybrid B16F1 mice were inoculated by i/v injection on d 0 with a sublethal dose of B16-BL6 tumor cells, and micrometastases were allowed to establish in the lungs. One day later, treatment with PROLEUKIN® by i/v injection was begun, using material diluted in either normal saline, D5W, or either vehicle with 0.1% HSA. PROLEUKIN® doses of 0.04, 0.1, 0.4, or 1 mg/kg/d were given daily for 4 days. At terminal sacrifice, lungs were removed from the animals, and the number of macroscopically evident metastases counted and compared between the groups. Six separate experiments, with 10 mice per group were conducted; the data presented in the table below represent results from the pooled information obtained from these studies.

TABLE III - Anti-Tumor Effects of PROLEUKIN® Delivered in Different Formulations

	Median Number of Metastases/Lung (Range)			
	Formulation Tested			
Dose of rhIL-2	Normal Saline	Saline + 0.1% HSA	D5W Only	D5W + 0.1% HSA
vehicle control	-n.d.-	72.5 (17, 212)	-n.d.-	109.5 (8, >250)
0.04 mg/kg	71.5 (11, > 250)	63.0 (3, > 250)	116.0 (8, >250)	92.5 (6, >250)
0.1 mg/kg	62.0 (1, > 250)	56.0 (5, > 250)	98.0 (5, > 250)	113.5 (15, > 250)
0.4 mg/kg	65.0 (21, 187)	47.0 (20, 156)	98.0 (6, > 250)	85.5 (17, > 250)
1.0 mg/kg	41.0* (1, 115)	45.0 (5, 187)	68.5* (2, 196)	70.5* (2, 192)

* significantly different from other groups, $p \leq 0.01$ (Kruskal-Wallace one-way ANOVA)

There was no significant anti-tumor activity of PROLEUKIN® in this disease model until the dose of 1.0 mg/kg was reached, although trends towards a dose-related decrease in the number of metastases were noted in all groups at doses of 0.4 mg/kg. More importantly, the addition of 0.1% HSA to the final formulation of PROLEUKIN® did not appear to affect the anti-tumor activity of the biologic; there were no significant differences in the median numbers or ranges of lung metastases in any of the HSA-containing rhIL-2 dose groups, when compared with the corresponding PROLEUKIN® dilutions without HSA.

In conclusion, the anti-tumor efficacy of PROLEUKIN® in the artificial, B16 micrometastasis model was not significantly impacted by either the choice of diluent (normal saline or D5W), nor by the addition of 0.1% HSA. These data support the proposed wording in the package insert regarding the dilution and delivery of PROLEUKIN® for treatment of metastatic melanoma or renal cell cancer.

Preclinical Pharmacokinetics Study Summary:

1. Quantitation of the renal clearance of PROLEUKIN® IL-2 using nephrectomized and ureter-ligated male CD rats. Study #PK/050994A. Crl:DD BR rats, weight range 250-450 g; 0.2, 0.5, 1.0 mg/kg rhIL-2, i/v bolus; non-GLP; final report dated 5/9/94; Chiron Corporation, Emeryville, CA. **Volume 4, pp. 217-296.**

2. Effect of human serum albumin (HSA) on the pharmacokinetics of PROLEUKIN® IL-2 in CD and Fischer rats. Study #INIL.298 and #IL.833. Males, weight range 0.25-0.45 kg, 4/dose

group; 0.2 mg/kg, 15 min i/v infusion (INIL.298) or 1.0 mg/kg, i/v bolus injection (IL.833); diluted in D5W or 0.1% HSA in D5W, held in Viaflex bags; non-GLP; final report dated 4/30/93; Chiron Corporation, Emeryville, CA. **Volume 2, pp. 329-396.**

3. Comparison of PROLEUKIN® IL-2 pharmacokinetics in rats following i/v bolus injection or 5-min infusion of a single 0.2 mg/kg dose. Study #PK051895A. Male, CD CRL1:DD BR rats, 6/group (weight range 500-600 gm); PROLEUKIN®; 0.2 mg/kg (lot # LAP-054) diluted in D5W; final report dated 2/15/97; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 4, pp. 397-455.**

Preclinical Pharmacokinetics Study Review:

Study #PK/050994A. ***Quantitation of the renal clearance of PROLEUKIN® IL-2 using nephrectomized and ureter-ligated male CD rats.***

As part of the phase 4 commitments to address the issue of whether impairment of renal function could affect PROLEUKIN® pharmacokinetics and toxicity, a preclinical study was conducted in male rats after either sham, single or double nephrectomy, or after a double ureter ligation. This study was designed to quantitate the contribution of renal clearance to the overall systemic clearance of rhIL-2, particularly in the setting of single nephrectomy, as would be expected in the case of renal cell carcinoma.

Male CD rats were cannulated in the jugular vein under ketamine anesthesia for the collection of blood samples prior to surgery. Functional single or double nephrectomy, or double ligation of the ureters were performed by ligation of either the renal pedicle(s) or the ureters approximately 1 to 2 h prior to PROLEUKIN® dosing. One group of animals received no surgical intervention, and served as the naïve control. Sham operations were performed similarly to the ligations described above, and served as additional controls.

Rats were dosed by a single, i/v bolus injection with PROLEUKIN® at doses of 0.2, 0.5, or 1 mg/kg. Samples of peripheral blood were collected from the cannulated jugular vein at various time points afterwards, and were analyzed for rhIL-2 concentration using a standard bioassay of murine T cell proliferation. Concentrations of rhIL-2 in samples from treated rats were determined from a standard curve of PROLEUKIN® diluted in pooled CD rat plasma, which was assayed concomitantly with the test materials. All pharmacokinetic calculations and modeling systems were generated using the PKDAAS program system on a VAX computer. Statistical analyses were performed using the SAS software package. Values were analyzed using a nonparametric Wilcoxon Exact test, with a Tukey's Studentized Range test performed to identify any groups significantly different from one another.

Comment: The data evaluated in the submission only reported values for clearance parameters (clearance, $t^{1/2}\alpha$, and $t^{1/2}\lambda$), and did not provide any information regarding rhIL-2 concentrations or exposure indices. The individual animal pharmacokinetic data were independently analyzed by this reviewer using the software program WinNonLin, and the statistical packages included in Microsoft Excel. One-way analysis of variance of the means and standard deviations for each parameter, with a Studentized t test to identify significant differences were used for the statistical analyses.

The results are presented in the table below. In rats with functional single nephrectomies, the C_{max}, elimination half-lives, and clearance of PROLEUKIN® after a 1.0 mg/kg dose were not statistically significantly different from the animals receiving either no surgery or the sham operation. The total exposure, as determined by the $AUC_{t0-\infty}$ was increased by approximately 2-fold over the control groups ($p \leq 0.01$). In those rats with double nephrectomies, PROLEUKIN® clearance was significantly decreased by almost 4-fold as compared to controls, and by 2.2-fold from that observed in the single nephrectomized animals ($p \leq 0.05$). Corresponding increases in AUC were also observed, with total exposures in double nephrectomized rats increased by approximately 4.5-fold when compared to the sham or naïve controls ($p \leq 0.01$) and by about 2-fold as compared to the single nephrectomized animals ($p \leq 0.05$). However, the elimination half-lives for PROLEUKIN® in single or doubly nephrectomized animals were not significantly different from either the sham or the naïve control groups; this finding is not explained by the current study design.

TABLE IV - Effects of Single or Double Nephrectomy on the Pharmacokinetics of PROLEUKIN® in CD and Fischer Rats

	Surgical Intervention			
P/K Parameter	**no surgery**	**sham nephrectomy**	**single nephrectomy**	**double nephrectomy**
Tmax (hr)	0.017 ± 0	0.017 ± 0	0.017 ± 0	0.017 ± 0
Cmax (ng/ml)	8709.6 ± 3688.2	8921.0 ± 2069.7	10097.1 ± 3724.9	7861.6 ± 1082.8
t1/2 elim (hr)	0.57 ± 0.08	0.59 ± 0.03	0.90 ± 0.35	1.24 ± 0.19
$AUC_{t0-\infty}$ (ng•hr/ml)	1293.9 ± 325.9	1410.3 ± 291.5	2691.8 ± 1654.4*	5362.0 ± 1431.7*,**
Clearance (ml/hr/kg)	811.3 ± 203.7	738.6 ± 190.5	457.5 ± 194.8	197.9 ± 57.3*,**
Vz init (ml/kg)	683.5 ± 247.9	636.2 ± 196.1	524.6 ± 120.8	357.8 ±137.0*,**
Vdss (ml/kg)	402.4 ± 216.2	303.8 ± 90.2	320.2 ± 66.2	340.0 ± 142.6

* significantly different from no surgery, sham controls, $p \leq 0.01$ (one-way ANOVA)
** significantly different from single nephrectomized, $p \leq 0.05$ (one-way ANOVA)

Similarly, ligation of both ureters resulted in significant, although smaller increases in AUC and decreases in systemic clearance of rhIL-2 when compared to sham-ligated control animals. The data are presented in the table below.

TABLE V - Effects of Double Ureter Ligation on IL-2 Pharmacokinetics

Surgical Intervention

P/K Parameter	sham ligation	double ligation
Tmax (hr)	0.017 ± 0	0.017 ± 0
Cmax (ng/ml)	8810.4 ± 2828.5	8231.6 ± 2223.67
t1/2 elim (hr)	0.55 ± 0.06	0.73 ± 0.22
$AUC_{t0-\infty}$ (ng•hr/ml)	1283.5 ± 348.3	1909.4 ± 257.9*
Clearance (ml/hr/kg)	789.4 ± 156.4	531.3 ± 75.0*
Vz initial (ml/kg)	649.7 ± 172.9	548.7 ± 104.0
Vdss (ml/kg)	296.3 ± 65.1	377.0 ± 155.3

* significantly different from sham ligation, p </= 0.01

In summary, the renal clearance of rhIL-2 in rats was significantly impaired after double nephrectomy, suggesting that this is the major route of elimination of PROLEUKIN®. Little effect on clearance or elimination half-life was noted after ligation of both ureters, suggesting that glomerular filtration is not a major mechanism of rhIL-2 clearance in the rat, but more likely clearance is related to active tubular secretion and degradation. Calculations by the sponsor have determined that the kidney is responsible for clearance of approximately 75% of a dose of rhIL-2 from the systemic circulation. However, no significant impairment of rhIL-2 clearance was noted after a single nephrectomy, suggesting that the pharmacokinetic and toxicity profiles in patients with renal cell cancer having undergone surgical resection of the tumor should be similar to those obtained in patients with intact renal function.

Study #INIL.298 and #IL.833. ***Effect of human serum albumin (HSA) on the pharmacokinetics of PROLEUKIN® IL-2 in CD and Fischer rats.***

Two studies were conducted by the sponsor to address the differential effects of addition of HSA on the pharmacokinetics of PROLEUKIN® in both CD and Fischer rats. In the first study, age-matched rats received a 0.2 mg/kg dose of rhIL-2 diluted in either D5W or D5W containing 0.1% HSA by a 15 minute i/v infusion. PROLEUKIN® was diluted to a final concentration of approximately 40 µg/ml in either vehicle, to match the recommended concentrations in use in the clinic. Rats were cannulated in the jugular vein under ketamine anesthesia for the collection of blood samples 24 h prior to dosing with rhIL-2. Samples of peripheral blood were collected from the cannulated jugular vein at various time points afterwards, and were analyzed for rhIL-2 concentration using a standard bioassay of murine T cell proliferation. Concentrations of rhIL-2 in samples from treated rats were determined from a standard curve of PROLEUKIN® diluted in pooled CD rat plasma, which was assayed concomitantly with the test materials. All pharmacokinetic calculations and modeling systems were generated using the PKDAAS program system on a VAX computer. Statistical analyses were performed using the SAS software package. Values were analyzed using a nonparametric Wilcoxon Exact test, with a Tukey's Studentized Range test performed to identify any groups significantly different from one another.

Comment: The data evaluated in the submission only reported values for clearance parameters (clearance, $t\frac{1}{2}\alpha$, and $t\frac{1}{2}\lambda$), and did not provide any information regarding rhIL-2 concentrations or exposure indices. The individual animal pharmacokinetic data were independently analyzed by this reviewer using the software program WinNonLin, and the statistical packages included in Microsoft Excel. One-way analysis of variance of the means and standard deviations for each parameter, with a Studentized t test to identify significant differences were used for the statistical analyses.

The results are presented in the table below.

TABLE VI - Effect of HSA on the Pharmacokinetics of PROLEUKIN® in CD and Fisher Rats After Slow Intravenous Infusion

	CD Rats		Fischer Rats	
P/K Parameter	**D5W Alone**	**0.1% HSA**	**D5W Alone**	**0.1% HSA**
Tmax (hr)	0.25 ± 0	0.25 ± 0	0.25 ± 0	0.25 ± 0
Cmax (ng/ml)	324.4 ± 42.8	386.0 ± 63.3	398.5 ± 134.9	679.8 ± 124.3*,**
t1/2 elim	0.25 ± 0.04	0.28 ± 0.04	0.27 ± 0.04	0.28 ± 0.02
$AUC_{t0-\infty}$ (ng•hr/ml)	95.7 ± 10.6	134.6 ± 25.6**	125.6 ± 38.7	214.5 ± 36.3*,**
Clearance (ml/hr/kg)	2109.8 ± 228.1	1524.1 ± 256.1**	1693.4 ± 414.4	955.8 ± 174.4*,**
Vz initial (ml/kg)	770.3 ± 180.5	608.6 ± 98.3	652.7 ± 129.9	387.3 ± 87.2
Vdss (ml/kg)	469.4 ± 140.3	392.6 ± 70.9	387.7 ± 96.4	221.7 ± 56.4

* significantly different from CD rats, $p \leq 0.03$
** significantly different with HSA added, $p \leq 0.01$

The data demonstrate that Fischer rats had consistently higher exposure ratios and slower clearance of rhIL-2, regardless of whether HSA was present in the formulation or not. With the addition of HSA to the PROLEUKIN® formulation, the mean value for C_{max} in the Fischer rats, but not the CD rats was increased by 70.6% as compared to the value obtained for rats of this strain treated with rhIL-2 diluted in D5W alone. Statistically significant increases in the AUC values of 40.6 and 74.1%, and corresponding decreases in clearance and V_d were obtained in both CD and Fischer rats, respectively, treated with material formulated with HSA.

The second study was conducted using similar methodology, but administering a higher dose (1.0 mg/kg rhIL-2) by i/v bolus injection. Age-matched, specific pathogen free CD and Fischer rats were used in the present study, and were cannulated in the jugular vein for sample collectio approximately 24 h prior to initiation of PROLEUKIN® treatment. PROLEUKIN® was reconsitituted from the lyophilized, vialed material with 1 ml of WFI, then further diluted in D5W to reach the final concentrations needed for dosing. Because the doses of PROLEUKIN® used in the present study could not be further diluted from the reconstituted material without significant protein precipitation and loss of bioactive material, concentrated HSA was injected immediately prior to PROLEUKIN® through a three-way stopcock. This is the same method of delivery used in the preclinical toxicology study described above. Samples of peripheral blood were collected at various time points after PROLEUKIN® injection, and the concentration of rhIL-2 in plasma determined as described above. The results are presented in the table below.

TABLE VII - Effect of HSA on the Pharmacokinetics of PROLEUKIN® IL-2 in CD and Fisher Rats by i/v Bolus Injection

P/K Parameter	CD Rats		Fischer Rats	
	D5W Alone	0.1% HSA	D5W Alone	0.1% HSA
Tmax (hr)	0.017 ± 0	0.017 ± 0	0.017 ± 0	0.017 ± 0
Cmax (ng/ml)	9981.5 ± 2496.7	8549.4 ± 1825.2	9183.64 ± 1581.2	12818.4 ± 1986.4*,**
t1/2 elim (hr)	0.20 ± 0.02	0.21 ± 0.02	0.20 ± 0.01	0.20 ± 0.02
$AUC_{t0-\infty}$ (ng•hr/ml)	1266.3 ± 187.2	1219.8 ± 105.4	1377.6 ± 113.7	1695.5 ± 484.8*
Clearance (ml/hr/kg)	802.0 ± 111.3	824.6 ± 73.5	729.8 ± 63.3	622.8 ± 155.8*
Vz initial (ml/kg)	241.0 + 44.2	259.1 ± 10.3	215.7 ± 17.0	179.4 ± 51.4*
Vdss (ml/kg)	190.75 ± 38.3	215.3 ± 16.6	185.6 ± 17.2	150.8 ± 35.7*

* significantly different from CD rats, p </= 0.03
** significantly different with HSA, p </= 0.02

After i/v bolus injection of PROLEUKIN®, only Fischer rats showed significant differences in pharmacokinetic parameters which could be related to the addition of HSA. The mean value for

C_{max} in this strain was increased by 39.6% with addition of HSA to the formulation. Similarly, the $AUC_{t0-\infty}$ was also increased in this strain only, with corresponding decreases in clearance and Vd. No significant changes in pharmacokinetic values for either C_{max}, clearance, Vd, or $AUC_{t0-\infty}$ were observed in CR rats which could be related to the addition of HSA. Fisher rats consistently demonstrated higher exposure ratios, and slower clearance than CD rats, whether or not HSA was present; however, these changes were not statistically significant unless HSA was present.

In conclusion, the addition of HSA to the dilutions of PROLEUKIN® used in the present two studies resulted in increased exposure to the drug, as demonstrated by the AUC values obtained after either slow i/v infusion or i/v bolus injection. Fischer rats consistently displayed higher AUC values and slower clearance or rhIL-2 than did CD rats, regardless of whether HSA was present in the formulation. Taken together, these data may offer a possible explanation of why the toxicity profile of PROLEUKIN® is different between these two strains of rats.

Study #PK051895A. ***Comparison of PROLEUKIN® IL-2 pharmacokinetics in rats following i/v bolus injection or 5-min infusion of a single 0.2 mg/kg dose.***

Because the previous two pharmacokinetic studies evaluating the effects of HSA on PROLEUKIN® biodistribution in Fischer and CD rats were conducted using different doses for the bolus and infusion regimens, the sponsor conducted a separate study in CD rats, designed to directly compare the pharmacokinetic profiles of rhIL-2 after administration of the same dose by either delivery method.

PROLEUKIN® doses of 0.2 mg/kg were administered to two groups of 6 CD rats by either i/v bolus injection or 5 minute, slow i/v infusion, respectively. Peripheral blood samples were collected at various time points after treatment, and rhIL-2 levels in the plasma determined by bioassay as described above. Using noncompartmental analysis methods, the pharmacokinetic profiles of PROLEUKIN® were obtained. The results are presented in the table below.

TABLE VIII - Comparison of PROLEUKIN® Pharmacokinetics in CD Rats Following i/v Bolus Injection or Five-Minute Infusion

P/K Parameter	I/V Bolus Injection	I/V Infusion
Tmax (hr)	0.01 ± 0.01	0.09 ± 0.01
Cmax (ng/ml)	2768.6 ± 457.7	670.9 ± 166.5*
t½ elim (hr)	0.97 ± 0.1	0.95 ± 0.1
$AUC_{t0-\infty}$ (ng•hr/ml)	307.3 ± 84.3	97.3 ± 17.9*
Clearance (ml/hr/kg)	688.2 ± 183.8	2118.1 ± 452.6*
Vz initial (ml/kg)	962.3 ± 265.8	2890.9 ± 670.5*
Vdss (ml/kg)	290.2 ± 58.2	707.6 ± 149.2*
Mean Residence Time (hr)	0.43 ± 0.04	0.34 ± 0.01

Following i/v bolus injection of 0.2 mg/kg PROLEUKIN®, significant differences were observed in several of the pharmacokinetic parameters as compared to those obtained following slow i/v infusion. Most notably, the mean values for C_{max} and $AUC_{t0-\infty}$ were increased in rats treated by i/v bolus injection by approximately 3-fold, as compared to those animals dosed with PROLEUKIN® by infusion ($p < 0.001$, Student's t-test). Both the clearance, as well as the initial volume of distribution and V_{dss} were increased by approximately 3-fold in the group of rats treated with PROLEUKIN® by i/v infusion, as compared to the values obtained in animals treated by bolus injection. However, the distribution half-lives (t½α and β, data not shown) and elimination parameters (t½λ, mean residence time) were not significantly different between the two methods of drug delivery.

Taken together, these data suggest that the higher initial plasma concentrations, total exposures, and slower clearance of PROLEUKIN® observed after bolus injection, as compared to slow i/v infusion may account for some of the differences in the toxicity profiles noted both in preclinical and clinical studies.

ADDITIONAL STUDIES SUBMITTED FOR INFORMATION ONLY (previously reviewed in PLA #88-0660)

Comment: Several study reports from preclinical safety and pharmacokinetic studies from the original application were also included in the submission, for informational and comparative purposes only. These data were reviewed at the time of the initial approval, and were not re-reviewed for the present application; a listing of the studies is provided below.

Preclinical Pharmacokinetics Study Summary:

1. Pharmacokinetic and allometric models of recombinant human interleukin-2 in mice, rats, rabbits, cynomolgus monkeys, and sheep. Study numbers ILM335, IL416, ILR7, PK-01-0189, and PS-01-0588. CD-1 mice, weight range 22-28 g, 3 ♂/time point, 7.5 mg/kg, i/v; CD rats, weight range 200-250 g, 1 mg/kg, i/v; New Zealand white rabbits, weight range 2.6, 2.7, 2.8 kg, 3 ♀, 3 mg/kg, i/v; cynomolgus monkeys, 1 each ♂ (3.7 kg) and ♀ (3.0 kg), 1 mg/kg, i/v; sheep (strain not specified), 3 ♀, weights 46.4, 49.1, 52.3 kg; 0.2 mg/kg, i/v; humans studies on file; final report date 12/19/90; non-GLP; Cetus/Chiron Corporation, Emeryville, CA. **Volume 2, pp. 15-109-A.**

2. The pharmacokinetics of PROLEUKIN® (aldesleukin) recombinant human interleukin-2 in CD-1 mice after intravenous bolus administration (0.25 mg/kg or 7.5 mg/kg) or subcutaneous administration (0.1 mg/kg or 1.0 mg/kg). Study # PK/042296A. CD-1 mice, (weight ranges 22.1-29.9 g, ♂ and 27.2-39.3 g, ♀); non-GLP; 9/13-11/21/88 (i/v) and 8/15-9/6/91; Cetus Corporation, Emeryville, CA. **Volume 2, pp. 110-216**.

3. Effect of dilution technique on the pharmacokinetics of PROLEUKIN® IL-2 in male CD rats. Study #INIL264 and #INIL-282. Male, Crl:DD BR rats, weight range 350-500 g, 3/dose group; 0.2 mg/kg/rat, 15 minute i/v infusion; diluted in D5W or 0.1% HSA in D5W, held in Viaflex bags vs. glass bottles; non-GLP; final report dated 3/27/92; Chiron Corporation, Emeryville, CA. **Volume 2, pp. 297-328.**

4. Effect of dilution technique on the recovery or PROLEUKIN® IL-2 in plasma of sheep given 0.04 mg/kg as a 15 minute infusion. Study #PS-01-0190. Final report dated 4/10/91; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 2, pp. 456-578.**

5. Sheep Study II. Effect of dilution technique on the pharmacokinetics of PROLEUKIN® IL-2 following a 15 minute infusion. Study #PK-01-0191. Final report dated 3/17/92; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 3, pp. 1-64.** - submitted to PLA #88-0660.

6. The pharmacokinetics of PROLEUKIN® (aldesleukin) recombinant human interleukin-2 in female sheep after intravenous bolus administration (0.01 mg/kg or 0.10 mg/kg) or subcutaneous administration (0.01 mg/kg or 0.1 mg/kg). Study #PK/042696A. Final report dated 4/26/96; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 3, pp. 65-222.**

7. A pharmacokinetic study of PROLEUKIN® recombinant interleukin-2 (IL-2) and polyethylene glycol-modified IL-2 (PEG IL-2) administered either subcutaneously or intravenously in female pigs with external thoracic duct-venous shunts for blood and lymph node sample collection. Study #PK/051796A. Final report dated 5/17/96; non-GLP; Chiron Corporation, Emeryville, CA. **Volume 3, pp. 223-373.**

Toxicology Study Summary:

1. Two-week intravenous infusion comparative toxicity study in the CD rat on PROLEUKIN® with or without 0.1% human serum albumin. Study #I01-91-007. **Volume 4, pp. 19-337.**

SUMMARY AND CONCLUSION:

Preclinical studies included in the present submission demonstrate that PROLEUKIN® is pharmacologically active in murine models of metastatic melanoma, at doses greater than or equal to 0.4 mg/kg/d, administered daily by i/v injection for 4 to 10 d. Dilution of PROLEUKIN® in vehicle containing 0.1% HSA had no significant effect on the anti-tumor activity of the agent. Dose- and schedule-related increases in mortality after PROLEUKIN® treatment were observed in mice administered 10 or 15 mg/kg/d for 7 to 14 d, which were further increased by the addition of HSA. Similar findings of increased toxicity and mortality were also observed in preclinical toxicity studies in rats treated with repeated doses of rhIL-2 treatment administered by i/v injection for 14 d, when HSA was present. Treatment-related toxicities included increases in total peripheral blood leukocyte, eosinophil and lymphocyte numbers, increases in total and indirect serum bilirubin and transaminase levels, and mortality. In outbred, Sprague-Dawley CD and inbred Fischer rats, no NOAEL could be determined. The lowest dose tested in CD rats was 0.5 mg/kg, and 2.0 mg/kg in the Fischer strain. Pharmacokinetic evaluation of PROLEUKIN® in these two strains of rats demonstrates that the presence of HSA in the diluent results in 23 to 35% increased in C_{max}, decreases in clearance and V_{dss}, and 40 to greater than 70% increases in total exposure, as defined by $AUC_{t0-\infty}$. Fifty to 200 percent greater values for $AUC_{t0-\infty}$ were achieved in Fischer rats as compared to CD rats when HSA was present, suggesting that the reasons for the increased mortalities observed in this strain using HSA in the diluent were related to the cumulative exposure to PROLEUKIN®. Direct comparison of a 5 min i/v infusion of 0.2 mg/kg PROLEUKIN® with the same dose delivered by i/v bolus injection demonstrated that the C_{max} and $AUC_{t0-\infty}$ values for IL-2 were increased approximately 3-fold in rats receiving the bolus injection as opposed to those animals treated by slow infusion.

Taken together, these data demonstrate that the previous increases in mortality noted in the preclinical studies using PROLEUKIN® in rats and mice are related to the presence of HSA in the formulation, and the schedule of delivery of the biologic. These data support the proposed labeling for the use of PROLEUKIN® formulations diluted without HSA, and administered by slow i/v infusion over a 5 to 15 min time period.

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Key Words: interleukin-2; metastatic melanoma; renal cell cancer; pharmacokinetics

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